Statement supplementing the environmental risk assessment conclusions and risk management recommendations on genetically modified insect-resistant maize 59122 for cultivation in the light of new scientific information on non-target organisms and regionally sensitive areas

Arpaia, Salvatore ; Birch, Nicholas ; Chesson, Andrew ; du Jardin, Patrick ; Gathmann, Achim ; Gropp, Jürgen ; Herman, Lieve ; Hoen-Sorteberg, Hilde-Gunn ; Jones, Huw ; Kiss, Jozsef ; Lovik, Martinus ; Messéan, Antoine ; Naegeli, H ; Nielsen, Kaare Magne ; Ovesna, Jaroslava ; Perry, Joe ; Rostoks, Nils ; Tebbe, Christoph

Abstract: Following a request from the European Food Safety Authority (EFSA), the Panel on Genetically Modified Organisms (GMO Panel) was asked to supplement its environmental risk assessment conclusions and risk management recommendations on maize 59122 for cultivation in the light of new scientific information on non-target organisms and regionally sensitive areas. Having considered additional information relevant to the assessment of potential adverse effects of maize 59122 on non-target organisms, the Panel must revise two of its previous environmental risk assessment conclusions, invalidating its earlier statement on the environmental safety of maize 59122 in its 2013 Scientific Opinion. A gap in the event-specific data on the honeybee study performed by Maggi (2001) was identified, as a result of which uncertainty over the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122 remains. Therefore, the Panel is no longer in a position to complete its assessment on the occurrence of adverse effects on pollinators. The Panel reassessed the available dataset on ladybirds, including the Califf and Ostrem (2009) study, and considers the latter study does not enable resolving the remaining scientific uncertainty on the potential toxicity of the binary Cry34Ab1/Cry35Ab1 proteins on Coccinella septempunctata or other ladybirds. In both cases, the Panel recommends that an additional laboratory study is performed prior to authorisation. The Panel considered regionally sensitive areas in its assessment, but regards it as premature to recommend specific risk management strategies for susceptible insect species potentially found within and nearby such areas owing to the inconclusive nature of the assessment of potential adverse effects of maize 59122 on non-target organisms

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SCIENTIFIC OPINION

Statement supplementing the environmental risk assessment conclusions and risk management recommendations on genetically modified insect-resistant maize 59122 for cultivation in the light of new scientific information on non-target organisms and regionally sensitive areas[1]

EFSA Panel on Genetically Modified Organisms (GMOs)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Food Safety Authority (EFSA), the Panel on Genetically Modified Organisms (GMO Panel) was asked to supplement its environmental risk assessment conclusions and risk management recommendations on maize 59122 for cultivation in the light of new scientific information on non-target organisms and regionally sensitive areas. Having considered additional information relevant to the assessment of potential adverse effects of maize 59122 on non-target organisms, the Panel must revise two of its previous environmental risk assessment conclusions, invalidating its earlier statement on the environmental safety of maize 59122 in its 2013 Scientific Opinion. A gap in the event-specific data on the honeybee study performed by Maggi (2001) was identified, as a result of which uncertainty over the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122 remains. Therefore, the Panel is no longer in a position to complete its assessment on the occurrence of adverse effects on pollinators. The Panel reassessed the available dataset on ladybirds, including the Califf and Ostrem (2009) study, and considers the latter study does not enable resolving the remaining scientific uncertainty on the potential toxicity of the binary Cry34Ab1/Cry35Ab1 proteins on *Coccinella septempunctata* or other ladybirds. In both cases, the Panel recommends that an additional laboratory study is performed prior to authorisation. The Panel considered regionally sensitive areas in its assessment, but regards it as premature to recommend specific risk management strategies for susceptible insect species potentially found within and nearby such areas owing to the inconclusive nature of the assessment of potential adverse effects of maize 59122 on non-target organisms.

KEY WORDS

GMO, *cry34Ab1*, *cry35Ab1*, insect resistance, non-target organisms, environmental safety, risk management

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[1] On request from EFSA, Question No EFSA-Q-2013-00607, adopted on 23 October 2013.

[2] Panel members: Salvatore Arpaia, Nicholas Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, Jozsef Kiss, Gijs Kleter, Martinus Lovik, Antoine Messéan, Hanspeter Naegeli, Kaare Magne Nielsen, Jaroslava Ovesna, Joe Perry, Nils Rostoks and Christoph Tebbe. Correspondence: gmo@efsa.europa.eu

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Genetically Modified Organisms (EFSA GMO Panel) to supplement its environmental risk assessment conclusions and risk management recommendations on maize 59122 for cultivation in the light of new scientific information on non-target organisms and regionally sensitive areas.

In its previous Scientific Opinion, the EFSA GMO Panel considered that maize 59122 is unlikely to have any adverse effect on the environment, except for the possible evolution of resistance to the Cry34Ab1/Cry35Ab1 proteins in coleopteran target pests. However, having considered additional information relevant to the assessment of potential adverse effects of maize 59122 cultivation on non-target organisms, the EFSA GMO Panel must revise two of its previous environmental risk assessment conclusions, invalidating its earlier statement on the environmental safety of maize 59122 in its 2013 Scientific Opinion (EFSA, 2013).

The EFSA GMO Panel has identified a gap in the data and considers that uncertainty over the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122 remains. Clarifications provided by the applicant at the request of the EFSA GMO Panel confirm that the *Bt*-maize pollen used as test substance in the honeybee study performed by Maggi (2001) is from an event other than maize 59122, and that the statements, with regard to the study, making reference to maize 59122 are incorrect in the application. Owing to the lack of event-specific data on plant–pollinator interactions, the EFSA GMO Panel is no longer in a position to complete its assessment on the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122, and must therefore invalidate its earlier statement on the environmental safety of maize 59122 in its 2013 Scientific Opinion (EFSA, 2013). To be able to conclude the environmental risk assessment of maize 59122, the EFSA GMO Panel recommends that the applicant supplies suitable event-specific data on plant–pollinator interactions prior to authorisation.

Following the publication of the additional advisory report on maize 59122 by the Netherlands Commission on Genetic Modification (COGEM), the EFSA GMO Panel reassessed the available dataset on ladybirds, including the Califf and Ostrem (2009) study. The latter study was found to be of limited value for the assessment of possible effects of the binary Cry34Ab1/Cry35Ab1 proteins on *Coccinella septempunctata* or other ladybirds, and therefore inappropriate to resolve the initially identified scientific uncertainty. To resolve the remaining scientific uncertainty on the potential toxicity of the binary Cry34Ab1/Cry35Ab1 proteins on ladybirds, the EFSA GMO Panel recommends that an additional laboratory study is performed to assess whether ladybirds are susceptible to the Cry34Ab1/Cry35Ab1 proteins. The EFSA GMO Panel considers it would be precautionary for a laboratory study to be conducted prior to authorisation.

The EFSA GMO Panel considered regionally sensitive areas in its assessment. However, owing to the inconclusive nature of the assessment of potential adverse effects of maize 59122 on non-target organisms, the EFSA GMO Panel regards it as premature to suggest specific risk management strategies for susceptible insect species potentially found within and nearby such areas.

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BACKGROUND AS PROVIDED BY EFSA

The EFSA GMO Panel issued a Scientific Opinion on application EFSA-GMO-NL-2005-23 for placing on the market of genetically modified (GM) maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003 (EFSA, 2013).

After the adoption of the Scientific Opinion in 2013, EFSA identified new information on non-target organisms and regionally sensitive areas relevant to the environmental risk assessment and risk management of maize 59122.

In the light of the newly identified scientific information, EFSA and its GMO Panel decided to review its environmental risk assessment conclusions and risk management recommendations on maize 59122 cultivation.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The EFSA GMO Panel is requested:

- to review its previous assessment of the potential adverse effects of maize 59122 cultivation on non-target organisms as well as associated risk management recommendations in the light of new relevant scientific information;

- to specify how regionally sensitive areas are accounted for in its environmental risk assessment conclusions and risk management recommendations on maize 59122 cultivation.

EVALUATION

1. Introduction

Maize 59122 provides: (1) protection against certain coleopteran target pests belonging to the genus *Diabrotica* such as the larvae of western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*Diabrotica barberi*) and southern corn rootworm (*Diabrotica undecimpunctata howardi*) through the expression of the binary Cry34Ab1/Cry35Ab1 proteins from *Bacillus thuringiensis* strain PS149B1; and (2) tolerance to the herbicidal active substance glufosinate-ammonium by the introduction of a gene coding for the enzyme phosphinothricin *N*-acetyltransferase (PAT) from *Streptomyces viridochromogenes.*

Maize 59122 is tolerant to glufosinate-ammonium-based herbicides, but the EFSA GMO Panel did not assess the potential adverse effects associated with the use of such herbicides on maize 59122, as maize 59122 will not be marketed in the EU as a herbicide-tolerant crop (EFSA, 2013).

2. Interactions of maize 59122 with non-target organisms

2.1. Adverse effects on non-target organisms due to unintended changes in maize 59122 (weight-of-evidence approach using event-specific *in planta* data)

2.1.1. Previous environmental risk assessment conclusions

The potential of maize 59122 to have adverse environmental effects on non-target organisms due to unintended changes in the GM plant was previously evaluated by the EFSA GMO Panel (EFSA, 2013). The EFSA GMO Panel reviewed molecular, compositional and agronomic/phenotypic data and all *event*-specific studies on main functional groups of non-target organisms such as (1) herbivores, (2) natural enemies, (3) pollinators and (4) decomposers.

Based on the evidence provided by the applicant and relevant scientific literature on maize 59122, the EFSA GMO Panel concluded that: "*there are no indications of adverse effects on non-target organisms due to unintended changes in maize 59122*" (EFSA, 2013).

The potential of maize 59122 to have adverse environmental effects on non-target organisms due to the expression of the binary Cry34Ab1/Cry35Ab1 proteins (intended changes in the GM plant) was previously evaluated by the EFSA GMO Panel (EFSA, 2013). Based on the toxicity data submitted by the applicant, the EFSA GMO Panel concluded that: "*no hazard to* Apis mellifera *is expected from exposure to plant-produced Cry34Ab1/Cry35Ab1 proteins*". Because there is no evidence to indicate that the cultivation of maize 59122 is likely to cause adverse effects on honeybees due to the expression of the Cry34Ab1/Cry35Ab1 proteins, this point is not further addressed in this EFSA GMO Panel Statement.

2.1.2. Additional information

In the application EFSA-GMO-NL-2005-23 for placing on the market of maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003, the applicant relied on the laboratory study with honeybee performed by Maggi (2001)[4] to exclude the occurrence of adverse effects on pollinators due to unintended changes in maize 59122.

Maggi (2001) reportedly fed individual honeybee larvae 2 mg pollen collected from either a Cry34Ab1/Cry35Ab1-expressing maize event or its near-isogenic counterpart. Larval survival was evaluated six and 12 days after treatment, and adult emergence was evaluated 26 days after treatment. No statistical differences in larval mortality between those fed Cry34Ab1/Cry35Ab1-expressing maize pollen and non-*Bt*-maize pollen were found, indicating that the honeybee development and survival were not affected by exposure to Cry34Ab1/Cry35Ab1-expressing maize pollen.

[4] Application EFSA-GMO-NL-2005-23: Technical dossier/section D.1/Annex 26: Maggi (2001).

However, EFSA, as part of its continuous process of screening all relevant scientific literature, has scrutinised a recent publication by the Center of Environmental Risk Assessment (CERA, 2013) that reviewed amongst others the laboratory study performed by Maggi (2001). The CERA (2013) monograph on the environmental safety of the Cry34Ab1/Cry35Ab1 proteins indicates that the pollen used as test substance by Maggi (2001) was from maize event TC5639 and not maize 59122 as claimed by the applicant (Table 2 in CERA, 2013).

Following a request from the EFSA GMO Panel, the applicant confirmed that the pollen used as test substance in the honeybee study performed by Maggi (2001) was from an event other than maize 59122, and that the statements, with regard to the study, making reference to maize 59122 were incorrect in the application.

The EFSA GMO Panel considers that event-specific data on plant–pollinator interactions are essential for the environmental risk assessment, and reiterates that, since unintended effects are to a large extent event specific, data from other events or from similar events in other plant species carry little weight in supporting an application (EFSA, 2010a,b).

Because no suitable event-specific data on plant–pollinator interactions are provided by the applicant, scientific uncertainty pertaining to the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122 remains. Therefore, the EFSA GMO Panel is no longer in a position to complete its assessment on the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122, and recommends that the applicant supplies suitable event-specific data on plant–pollinator interactions prior to authorisation.

2.1.3. New scientific literature

In the course of the routine literature searches performed by EFSA to identify new information on effects of GM plants on non-target organisms, no additional new peer-reviewed publications of relevance to maize 59122 were found in the period March to October 2013.

2.1.4. Conclusion

The EFSA GMO Panel notes that the pollen used as test substance in the honeybee study performed by Maggi (2001) was from an event other than maize 59122, and that the statements, with regard to the study, making reference to maize 59122 were incorrect in the application. The EFSA GMO Panel has identified a gap in the data and considers that uncertainty over the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122 remains. Therefore, the EFSA GMO Panel is no longer in a position to complete its assessment on the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122, and must therefore invalidate its earlier statement on the environmental safety of maize 59122 in its 2013 Scientific Opinion (EFSA, 2013). To be able to conclude the environmental risk assessment of maize 59122, the EFSA GMO Panel recommends that the applicant supplies suitable event-specific data on plant–pollinator interactions prior to authorisation.

2.2. Adverse effects on non-target organisms due to the expression of the binary Cry34Ab1/Cry35Ab1 proteins (tiered approach)

2.2.1. Previous environmental risk assessment conclusions

The potential of maize 59122 to have adverse environmental effects on non-target organisms due to the expression of the binary Cry34Ab1/Cry35Ab1 proteins (intended changes in the GM plant) was previously evaluated by the EFSA GMO Panel (EFSA, 2013).

2.2.1.1. Non-target organisms in general

In its Scientific Opinion on maize 59122 cultivation, the EFSA GMO Panel considered that: "*the potential adverse effects of maize 59122 due to the expression of the Cry34Ab1, Cry35Ab1 and PAT proteins on non-target terrestrial (plant- and ground-dwelling), soil and aquatic arthropods, as well as non-target organisms that are not arthropods, are expected to be negligible in the context of its intended uses, except for chrysomelids. The risk of maize 59122 to non-target chrysomelid species in the field is low due to their low occurrence and abundance in maize fields and because of the low likelihood of encountering harmful amounts of pollen from maize 59122 in and around maize fields. Non-target adult chrysomelids, which may occasionally feed on maize 59122 plants, are not expected to be affected due to the low activity of the Cry34Ab1/Cry35Ab1 proteins on adults. Furthermore, the only protected chrysomelid species (*Macroplea pubipennis*) considered to be at risk across the EU (under Directive 92/43/EEC on conservation of natural habitats and of wild fauna and flora) does not occur in maize fields*". In addition, "*no risk to non-target Lepidoptera is expected from exposure to maize 59122 pollen in the field*" and "*no hazard to* Culex quinquefasciatus *and no risk to Diptera are expected from exposure to plant-produced Cry34Ab1/Cry35Ab1 and maize 59122*". With regard to soil microorganisms and microbial communities, "*potential effects due to the cultivation of maize 59122, if they occur, will be transient and minor, and are likely to be smaller or within the range currently caused by other agronomic and environmental factors*" (EFSA, 2013).

2.2.1.2. Coccinellidae (ladybirds)

In its Scientific Opinion on maize 59122 cultivation, the EFSA GMO Panel considered that: "*Cry34Ab1/Cry35Ab1 proteins may be toxic to* Coleomegilla maculata *at dose levels that exceed field exposure. However, adverse effects were not seen at field dose levels when* C. maculata *larvae were fed a mixture of natural prey and pollen*". Therefore, the EFSA GMO Panel is of the opinion that: "*there is reasonable certainty that maize 59122 will not adversely affect* C. maculata". However, the GMO Panel noted: "*possible sublethal effects on* C. maculata *at Cry34Ab1/Cry35Ab1 dose levels that exceed field exposure, as well as limitations in the experiments*" and therefore, to resolve the remaining uncertainty, it requested additional data on a representative European coccinellid species. In response, the applicant provided two types of lower-tier studies (including tri-trophic experiments) with the focal species *Coccinella septempunctata*. Based on the submitted toxicity data and estimated worst-case expected environmental concentrations, the EFSA GMO Panel concluded that: "*no hazard to* C. septempunctata *and no risk to coccinellids are expected from exposure to plant-produced Cry34Ab1/Cry35Ab1 proteins or maize 59122*". Therefore, the EFSA GMO Panel considered that: "*case-specific monitoring of coccinellids is not necessary*" (EFSA, 2013).

2.2.2. **Additional information**

In its recent advisory report on the cultivation of maize 59122, the Netherlands Commission on Genetic Modification (COGEM) assessed whether the additional information on ladybirds provided by the applicant upon request of the EFSA GMO Panel gave reason to reconsider its previous recommendation for the necessity for case-specific monitoring of coccinellids (COGEM, 2013). In its previous advice, COGEM was of the opinion that: "*monitoring of ladybirds is necessary because in laboratory experiments exposure of ladybird larvae to high levels of Cry34Ab1/Cry35Ab1 proteins resulted in a reduced larval weight, which may affect the rate of reproduction*" (COGEM, 2008). Because in the additional study with the European ladybird *C. septempunctata* performed by Califf and Ostrem (2009)[5] larvae were exposed to low or unknown levels of Cry34Ab1/Cry35Ab1 proteins, COGEM considered: "*it is impossible to draw conclusions on the absence or presence of an effect*". Therefore, the additional information did not remove COGEM's previous concerns, and COGEM was of the opinion that: "*case-specific monitoring of ladybirds remains a necessity, unless conclusive additional experimental data is provided showing unequivocally the absence of an effect*".

[5] Application EFSA-GMO-NL-2005-23: Additional information received on 27/01/2010/Request 1/pages 1–3/Annex 2: Califf and Ostrem (2009)/Annex 3: Hong (2009).

Following the publication of COGEM's additional advisory report, the EFSA GMO Panel reanalysed the available dataset on coccinellids, including the laboratory study with *C. septempunctata* performed by Califf and Ostrem (2009).

- In the laboratory experiment by Califf and Ostrem (2009), larvae were fed a diet composed of lyophilised maize 59122 pollen mixed and ground with lepidopteran eggs of *Ephestia kuehniella* at a 1:3 ratio by weight. No statistically significant differences in survival, development time or adult weight were identified when compared with the corresponding non-*Bt*-maize control. Adult weight of *C. septempunctata* in the study was low compared with that of ladybirds fed optimal food (such as aphids). "*Quantitative ELISA measured levels of Cry34Ab1 protein at 2.8 ng/mg (dry weight) in 59122 maize pollen*" (Califf and Ostrem, 2009). The level of the Cry35Ab1 protein in maize pollen was not determined in the Califf and Ostrem (2009) study, because this level was found to be low (< 0.324 µg/g (dry weight)) or below the level of quantification in maize 59122 pollen (reviewed by CERA, 2013; COGEM, 2013; EFSA, 2013).

  The EFSA GMO Panel notes that the level of Cry34Ab1 protein in maize 59122 pollen collected from European field trials (with three locations in Bulgaria (2003 and 2004) and three locations in Spain (2004)) in 2003 and 2004 ranged between 45.5 and 146 µg/g (dry weight). In addition, the highest reported Cry34Ab1 concentration in maize 59122 pollen according to CERA (2013) was 87.2 µg/g (dry weight). Finally, the biological activity of the Cry34Ab1/Cry35Ab1 proteins in thawed pollen was not determined at diet administration. The EFSA GMO Panel therefore agrees with COGEM that, because of the low or unknown levels of exposure to the plant-produced Cry34Ab1/Cry35Ab1 proteins, the Califf and Ostrem (2009) study is of limited value for the assessment of possible effects of the binary Cry34Ab1/Cry35Ab1 proteins on *C. septempunctata* or other ladybirds.

- In the tri-trophic experiments, *C. septempunctata* larvae were fed among others a diet consisting of maize 59122 pollen only, or a mixture of maize 59122 pollen and aphids (*Rhopalosiphum padi*) reared on maize 59122.[6] Results indicated no statistically significant differences between larval development, adult weight and survival of *C. septempunctata* between the pollen only or pollen–aphid and corresponding non-*Bt*-control treatments (Takács et al., 2010). Because the expression level of the Cry34Ab1/Cry35Ab1 proteins and their bioactivity in maize 59122 pollen were not determined, the study results are difficult to interpret (COGEM, 2013).

  In another tri-trophic study in which aphids reared on maize 59122 were used as the sole food source for *C. septempunctata*, no significant differences were observed during the test period in either fecundity or fertility of *C. septempunctata* across the *Bt*- and non-*Bt*-based treatments (Takács et al., 2012). Aphids reared on maize 59122 were the sole food source for *C. septempunctata*, although they do not represent a significant route of exposure to Cry34Ab1/Cry35Ab1. Because exposure to Cry34Ab1 via aphids is likely to be negligible, the study does not allow any conclusions to be drawn about the potential adverse effects of Cry34Ab1/Cry35Ab1 on the fecundity or fertility of *C. septempunctata*.

  The limited value of the tri-trophic experiments in reducing the remaining scientific uncertainty was previously pinpointed by the EFSA GMO Panel (EFSA, 2013).

[6] Application EFSA-GMO-NL-2005-23: Additional information received on 27/01/2010/Request 2/page 4/Annex 5: Hungarian Academy of Sciences Plant Protection Institute (undated).

- Field trials performed in the United States in the growing seasons 2004–2005[7] and 2005–2007[8], in Spain in the growing seasons 2005–2007[9] and in Hungary in the growing seasons 2006–2008[10] suggested the absence of adverse effects of maize 59122 on ladybirds. However, ladybirds were generally collected in low numbers in these field trials. Therefore, results of these field trials should be cautiously interpreted to draw any conclusions about possible effects of the plant-produced Cry34Ab1/Cry35Ab1 proteins on ladybirds (see also COGEM, 2013). Consequently, the EFSA GMO Panel must revise its initial opinion that incorrectly stated that the field trials delivered comprehensive data for coccinellids.

- Vinall (2011)[11] assessed the potential adverse effects of maize pollen containing 59122 × 1507 × NK603 on *C. septumpunctata* survival and adult weight under laboratory conditions. The maize pollen containing 59122 × 1507 × NK603 was mixed with freeze-killed eggs of *E. kuehniella*, in a 1:3 ratio. Ladybirds fed the test diet containing maize 59122 × 1507 × NK603 pollen were compared with those fed the control diet. No relevant differences in survival or adult weight were observed. Although the study performed by Vinall (2011) suggests no adverse effects on *C. septumpunctata* following exposure to maize 59122 × 1507 × NK603 pollen, the EFSA GMO Panel did not use it as bridging study to support the risk assessment of maize 59122, as the Panel considers the risk assessment of single events a prerequisite for the risk assessment of stacked events.

The EFSA GMO Panel agrees with COGEM that scientific uncertainty pertaining to the potential toxicity of the Cry34Ab1/Cry35Ab1 proteins on ladybirds remains. To resolve the remaining scientific uncertainty, the EFSA GMO Panel recommends that an additional laboratory study is performed to assess whether ladybirds are susceptible to the Cry34Ab1/Cry35Ab1 proteins. Field studies are not considered the most optimal study type in this case, as in-field exposure to the plant-produced Cry34Ab1/Cry35Ab1 proteins can be limited: maize pollen shed lasts for approximately two weeks; aphids contain no or negligible levels of plant-produced Cry34Ab1/Cry35Ab1 proteins; the occurrence and abundance of ladybirds in maize fields is limited. Therefore, it would be challenging to detect potential sublethal adverse effects under field conditions, especially if abundance is used as sole measurement endpoint.

The EFSA GMO Panel is of the opinion that a laboratory study in which larvae of a EU ladybird species are exposed to the pure Cry34Ab1/Cry35Ab1 proteins at the maximum hazard dose for a suitable test duration would deliver robust data, provided that the following conditions are met: the purity of the Cry34Ab1/Cry35Ab1 proteins used as test substance is high (almost 100 %); the stability and biological activity of the test substance is confirmed; exposure of the test organisms to the test substance is confirmed; sufficient individuals are tested to ensure sufficient power to detect an effect were it to exist; negative control treatments are included to assess the suitability of the test system; positive control treatments are included, where feasible, to demonstrate that the test system is able to detect both lethal and sublethal treatment effects, using relevant endpoints (including survival, growth, development, egg production and hatch rate) (EFSA, 2010; Romeis et al., 2011).

Alternatively, a tri-trophic study with a spider mite-consuming ladybird (i.e. ladybirds belonging to the Stethorini tribe such as *Stethorus punctillum*) could be performed, as spider mites have been shown to contain plant-produced Cry protein concentrations that are similar to those measured in the leaves on which they have fed. Furthermore, bioassays demonstrated that plant-produced Cry proteins contained

[7] Application EFSA-GMO-NL-2005-23: Additional information received on 16/01/2012/Annex: Higgins and Dively (2006).
[8] Application EFSA-GMO-NL-2005-23: Additional information received on 16/01/2012/Annex: Higgins et al. (2009).
[9] Application EFSA-GMO-NL-2005-23: Additional information received on 20/12/2007/Annexes 1 and 3: Higgins and Hong (2007)//27/01/2010/Annex 9: Pascual and Hong (2008)//16/01/2012/Annex: Higgins and Hong (2008a).
[10] Application EFSA-GMO-NL-2005-23: Additional information received on 20/12/2007/Annex 2: Higgins and Hong (2007)//27/01/2010/Annex 8: Pascual and Hong (2008)//16/01/2012/Annexes: Higgins and Hong (2008b) and Pascual (2010).
[11] Application EFSA-GMO-UK-2006-30: Additional information received on 04/06/2013/Annex: Vinall (2011b).

in spider mites after feeding on *Bt*-maize retain their biological activity (Romeis et al., 2011, and references therein).

The EFSA GMO Panel considers it would be precautionary for a laboratory study to be conducted prior to authorisation.

2.2.3. New scientific literature

In the course of the routine literature searches performed by EFSA to identify new information on effects of GM plants on non-target organisms, no additional new peer-reviewed publications of relevance to maize 59122 were found in the period March to October 2013.

2.2.4. Conclusion

Following the publication of COGEM's additional advisory report on maize 59122 (COGEM, 2013), the EFSA GMO Panel reassessed the available dataset on ladybirds, including the Califf and Ostrem (2009) study. The latter study was found to be of limited value for the assessment of possible effects of the binary Cry34Ab1/Cry35Ab1 proteins on *C. septempunctata* or other ladybirds, and thus inappropriate to resolve the remaining scientific uncertainty. The EFSA GMO Panel therefore must revise its previous environmental risk assessment conclusions on this specific matter.

To resolve the remaining scientific uncertainty on the potential toxicity of the binary Cry34Ab1/Cry35Ab1 proteins on ladybirds, the EFSA GMO Panel recommends that an additional laboratory study is performed to assess whether ladybirds are susceptible to the Cry34Ab1/Cry35Ab1 proteins. Field studies are not considered the optimal study type in this case; instead the EFSA GMO Panel advocates a laboratory study in which larvae of a EU ladybird species are exposed to the pure *Bt*-proteins at the maximum hazard dose for a suitable test duration or a tri-trophic study with a spider mite-consuming ladybird. The EFSA GMO Panel considers it would be precautionary for a laboratory study to be conducted prior to authorisation.

3. Consideration of regionally sensitive areas

3.1. Introduction

Greiter et al. (2013) identified regionally sensitive areas in Austria and developed justifications for respective cultivation restrictions and prohibitions for maize 59122 in those areas.

3.2. Assessment

Article 6(5e) of Regulation (EC) No 1829/2003 requires, where applicable, conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements, to be based on the outcome of the risk assessment and, in the case of GMOs or food containing or consisting of GMOs, conditions for the protection of particular ecosystems/environments and/or geographical areas. The EFSA GMO Panel therefore considered the scientific rationale put forward by Greiter et al. (2013) to justify cultivation restrictions and prohibitions for maize 59122 in regionally sensitive areas in the light of plausible scenarios of harm described in the EFSA GMO Panel Scientific Opinion on maize 59122.

Survival of maize outside cultivation in Europe is limited by a combination of low competitiveness, the absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climatic conditions. Furthermore, as these general characteristics are unchanged in maize 59122, it is considered very unlikely that it or its progeny will differ from conventional maize varieties in their ability to establish feral populations under European environmental conditions. The insect resistance and herbicide tolerance traits are not likely to provide selective advantages outside cultivation or other areas where glufosinate-ammonium-based herbicides could be applied in Europe. Therefore, maize 59122 plants are not likely to establish feral populations under European environmental conditions including regionally sensitive areas.

Although maize 59122 is tolerant to glufosinate-ammonium-based herbicides, it will not be marketed in the European Union as a herbicide-tolerant crop. Therefore, potential adverse effects associated with the use of such herbicides on maize 59122 were not considered (see EFSA (2013) for more details).

A possible route through which maize 59122 may harm the environment is via exposure through Cry34Ab1/Cry35Ab1-expressing plant material. Depending on the seriousness and the likelihood of harm arising from the potential cultivation of maize 59122 within and nearby regionally sensitive areas, specific risk management strategies for non-target organisms could be foreseen to limit exposure of susceptible species potentially found within and nearby such areas (EFSA, 2011, 2012). However, owing to the remaining scientific uncertainty on the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122 and on the potential toxicity of the binary Cry34Ab1/Cry35Ab1 proteins on ladybirds, the EFSA GMO Panel regards it premature to suggest specific risk management strategies for susceptible insect species potentially found within and nearby such areas. The necessity for specific risk management strategies for susceptible insect species found within and nearby regionally sensitive areas will therefore be explored fully only once the missing data have been provided.

3.3. Conclusion

The EFSA GMO Panel considered regionally sensitive areas in its assessment, but regards it as premature to recommend specific risk management strategies for susceptible insect species potentially found within and nearby such areas owing to the inconclusive nature of the assessment of potential adverse effects of maize 59122 on non-target organisms.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Having considered additional information relevant to the assessment of potential adverse effects of maize 59122 cultivation on non-target organisms, the EFSA GMO Panel must revise two of its previous environmental risk assessment conclusions, invalidating its earlier statement on the environmental safety of maize 59122 in its 2013 Scientific Opinion (EFSA, 2013).

A gap in the event-specific data on the honeybee study performed by Maggi (2001) was identified, as a result of which uncertainty over the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122 remains. Therefore, the EFSA GMO Panel is no longer in a position to complete its assessment on the occurrence of adverse effects on pollinators due to potential unintended changes in maize 59122, and must therefore invalidate its earlier statement on the environmental safety of maize 59122 in its 2013 Scientific Opinion (EFSA, 2013). To be able to conclude the environmental risk assessment of maize 59122, the EFSA GMO Panel recommends that the applicant supplies suitable event-specific data on plant–pollinator interactions prior to authorisation.

The Califf and Ostrem (2009) study was found to be of limited value for the assessment of possible toxic effects of the binary Cry34Ab1/Cry35Ab1 proteins on *C. septempunctata* or other ladybirds, and therefore inappropriate to resolve the remaining scientific uncertainty. To resolve the remaining scientific uncertainty, the EFSA GMO Panel recommends that an additional laboratory study is performed to assess whether ladybirds are susceptible to the Cry34Ab1/Cry35Ab1 proteins. The EFSA GMO Panel considers it would be precautionary for a laboratory study to be conducted prior to authorisation.

The EFSA GMO Panel considered regionally sensitive areas in its assessment, but regards it as premature to recommend specific risk management strategies for susceptible insect species potentially found within and nearby such areas owing to the inconclusive nature of the assessment of potential adverse effects of maize 59122 on non-target organisms.

DOCUMENTATION PROVIDED TO EFSA

1. Note, dated 31 May 2013, from the Chair of the EFSA GMO Panel to the EFSA Executive Director to request a self-task mandate to supplement the environmental risk assessment conclusions and risk management recommendations on maize 59122 for cultivation.

2. Letter from EFSA to the applicant, dated 31 May 2013, requesting clarifications.

3. Letter from the applicant to EFSA, dated 22 June 2013, providing the clarifications requested by EFSA.

4. Acknowledgement letter, dated 28 June 2013, from the EFSA Executive Director to the Chair of the EFSA GMO Panel.

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